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PRENATAL NUTRITION OF POULTRY AND ITS POSTNATAL EFFECTS
(review)

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Abstract

Fast growth rate in modern meat-type poultry is accompanied by several metabolic disorders resulting from the discrepancy between embryonic and postembryonic growth and development. Prenatal period of avian ontogenesis is characterized by abrupt physiologic and metabolic alterations and hence any disturbance at this stage can affect the hatch efficiency and subsequent postnatal growth and productivity (E.T. Moran, 2007; V.L. Christensen et al., 2004). The embryonic bird development can be supported by the in ovo nutrition using natural nutrients (amino acids, carbohydrates, vitamins) as well as growth stimulators and hormones; this approach can also prepare the poults for the intense postnatal growth (P.R. Ferket, 2016). From the nutrigenomic point of view, the nutrients and bioactive substances can affect gene expression (V.I. Fisininet al., 2006; L. Bordoniet al., 2019). The experiments with the in ovo nutrition proved that the injections of nutrients can affect physiological status of broiler embryos and hatched broiler chicks. E.g. the injections of carbohydrates enlarge the pool of energy available for the embryo and decreases the catabolism of proteins and lipids during the final stage of incubation, resulting in the increases in the weight of the hatched chicks and in postnatal growth rate, supported by better development of the gastrointestinal tract (R. Kornasioet al., 2011; R. Jhaet al., 2019). All amino acids are necessary for the developing embryo; the absence of any of the amino acids can disrupt protein synthesis and homeostasis in the embryo, resulting in poorer postnatal growth and development. A bulk of studies were published which demonstrated the positive effects of the in ovo injections of individual amino acids and their combinations on the postnatal growth rate (Y. Ohtaet al., 2001; T.M. Shafeyet al., 2014; L.L. Yu et al.,2018). Ca. 94% of total metabolizable energy in the embryo is generated via the oxidation of fatty acids. These oxidative processes, in turn, generate substantial amounts of free radicals which can result in vast cellular damage (P. Surai and V.I. Fisinin, 2013; A. Yigitet al., 2014). The administration of vitamins with antioxidative activity (like C or E) during the embryonic period positively affected the postnatal development of the immune system in chicks (S.A. Selimet al., 2012; S. Nowaczewskiet al., 2012). The administration of L-carnitine into the embryos was shown to enhance pre-hatch glucose utilization in the anaerobic conditions and postnatal growth in the chicks (T.M. Shafeyet al., 2010; A.M. Dolgorukova, 2017). The in ovo nutrition can therefore be an instrument of significant improvement of the hatchability of the eggs and subsequent growth efficiency in hatched chicks, resulting in explicit economic effect (E.D. Peebles, 2018). It should, however, be noted that this technique has not still found application in the commercial poultry production and that for wider knowledge on the stimulating effects of different nutrients on the development of avian embryo further research is required.

Keywords: embryonic development, broilers, prenatal period, in ovo feeding, amino acids, antioxidants, vitamin E, vitamin C, L-carnitine, growth rate

Poultry farming is one of the leading branches of agriculture for producing relatively cheap and biologically complete food products. The peculiarities of the reproduction of domesticated birds, in particular the fact that the efficiency of reproduction in birds is definitely higher than in mammals, led to the emergence of industrial poultry farming [1].

Over the past 50 years, significant progress has been made in improving the functional characteristics of poultry through genetic selection. Thus, from 1957 to 2005, the growth rate of broilers increased by about 400% (for 5-6 weeks of life). At the same time, a higher body weight is achieved in less time with a decrease in feed consumption per 1 kg of body weight gain [2, 3]. However, such rapid growth and development caused a number of adverse complications, including ascites, skeletal abnormalities, immunosuppression, as well as increased susceptibility to infectious diseases [4]. According to Buzała et al. [5], such metabolic complications can be caused by inconsistencies between the processes of growth and development during embryogenesis and in the postembryonic period [5]. In modern broiler breeds and crosses, the duration of embryonic and neonatal development reaches 50% of the period of productivity (35-42 days of life).

Genetic selection determines the genotype of poultry; however, how inherited genes are expressed depends on external conditions – nutrition and growing technology. According to modern concepts of nutrigenomics, nutrients and biologically active substances can influence gene expression [6, 7]. These factors have the greatest effect during periods of intensive cell division while embryogenesis, leading to permanent effects throughout the entire postnatal period [8, 9]. The nature of nutrients supplied to the chick during the prenatal period is formed by the so-called food (or epigenetic) imprinting, on which the further growth and development of the organism depend. During epigenetic imprinting, DNA methylation occurs in the regions of promoters of specific genes, which can modulate for a long time the adaptive response of the organism to various stimuli during critical periods of development [10]. In poultry, epigenetic programming can be conducted during two critical periods –in a young industrial stock during gametogenesis and during embryogenesis, when egg nutrients are consumed by the embryo through the amniotic fluid and yolk [11].

The embryonic development of poultry can be supported and the chicks can be better prepared for intensive growth by using *in ovo* nutrition with natural nutrients — amino acids, carbohydrates, vitamins, as well as growth stimulants and hormones. The purpose of this review is to analyze the data available in the literature on the effects of carbohydrates, amino acids, antioxidants, and vitamins, when administered *in ovo*, on the embryonic and post-embryonic development of poultry, on the possible mechanisms of these effects, as well as on the prospects for creating industrial technologies of prenatal nutrition.

Technology of prenatal *in ovo* nutrition. The last period of incubation is characterized by oral intake of amniotic fluid by the embryo, intensive resorption of the yolk, accumulation of glycogen stores in muscles and liver for their use during pecking and hatching, the onset of pulmonary respiration, retraction of the residual yolk into the abdominal cavity, and finally, after pecking, the chick comes out of the shell [12, 13]. During this period, sharp physiological and metabolic changes occur, and any arising disturbances (for example, delay in nutrient use, incubation temperature) affect hatching efficiency and subsequent productivity [14-17].

The experiments using *in ovo* nutrition technology have shown that injections of nutrients affect the physiological state of broiler and chick embryos after hatching. The nutrients and biologically active substances introduced in this way ultimately improve the nutritional status of the chicks, which leads to a greater growth potential [18-20]. The injection site mainly depends on the age of the embryo. Thus, Ebrahimi et al. [18] propose to introduce substrates into egg albumen to a depth of 12 mm before incubation, as well as at its initial stage. After 17 days of incubation, the injections are carried out into other parts of the egg —

the air chamber and amnion. For the introduction of exogenous sources of nutrients and biologically active substances *in ovo*, either insulin syringes are used (under laboratory conditions) [21] or installations that ensure the processing of a large batch of eggs. The needles used in such devices are designed to reach the amnion [22].

Features of carbohydrate metabolism and the use of their exogenous sources during embryogenesis. One of the main physiological processes during the hatching period is the maintenance of glucose homeostasis. The liver plays a central role in carbohydrate metabolism and the delivery of glucose to tissues during embryogenesis, performing glucose synthesis from non-carbohydrate precursors (gluconeogenesis), glycogen synthesis (glycogenesis), and glycogen breakdown (glycogenolysis) [23, 24]. Glycogen stores are consumed as embryos go through the hatching process [25]. The high growth rate of the embryo is associated with high energy consumption. The glycogen stores in the liver and muscles are unable to meet the metabolic needs of the embryo, especially on the last day of embryogenesis. Low glycogen content in the liver correlates with longer hatching and lower body weight at hatching [14]. For homeostatic regulation of the amount of glucose in the blood, the embryo is forced to generate energy using various metabolic processes, for example, gluconeogenesis using glycerol as a substrate, released after lipolysis, or an amino acid after proteolysis [13]. Proteolysis, during which protein degradation is conducted, negatively affects the development of embryos [26, 27]. Since there are no carbohydrates in the egg, glycogen stores will begin to replenish only when the hatched chick has full access to the feed [13].

The introduction of different types of carbohydrates *in ovo* is likely to increase the amount of available energy for the embryo and reduce the catabolism of proteins and lipids during internal hatching. Zhai et al. [22] showed that injections of carbohydrates (glucose, sucrose, maltose, and dextrins, 0.25 g of active ingredient per 1 ml of diluent) statistically significantly ($p \leq 0.001$) increased the body weight of chicks while hatching, which increased in direct proportion to the volume of the injected solution. The authors recommend injecting no more than 0.4 ml of sucrose and 0.7 ml of glucose, maltose, and dextrin, while maintaining 90% hatchability. Fructose, unlike other carbohydrates, reduced hatchability and the body weight of chicks. The authors did not explain the reason for this [22].

At the end of the incubation period, rapid growth and maturation of visceral organs occur [28, 29]. Over the last 6 days of incubation, the area of the absorption surface in the small intestine increases 5-fold, the number of enterocytes increases, goblet cells appear that produce acidic mucin, and the ability to digest and absorb develops [30]. The earlier the intestines reach functional maturity, the faster the chicks will be able to use the nutrients in the feed, effectively absorb minerals and vitamins, thereby supporting the development of the most important organs and systems (skeleton, immune system, pectoral muscle). The observed increase in the body weight of chicks receiving carbohydrates during embryonic development may be associated with improvement in the development of the gastrointestinal tract, which was confirmed in the study by Kornasio et al. [19]. In chicks which received a mixture of dextrin and hydroxymethyl butyrate during the embryonic period, the glycogen content in the liver and muscles increased and the proliferation of satellite cells of muscle tissue was activated. The effect of the solution, injected *in ovo*, was long-lasting and influenced the weight of the poultry at the end of the rearing period [19]. Salmanzadeh et al. [31] showed that chicks that received a mixture of glucose and magnesium during the embryonic period had a greater body weight at hatching and on the 42nd day of life compared with the control group; in addition, the slaughter yield and the yield of pectoral muscles increased [31]. Similar results were

obtained in the authors' experiments. In 1-day-old chicks of meat-type mini chicken of the B77 line, the relative mass of the glandular and muscular stomachs in the group receiving dextrin in the prenatal period was significantly higher than in the control intact group. The live weight of 21-day-old chicks from the experimental groups, injected *in ovo* with glucose and dextrin (0.5 ml of a 10% solution), exceeded that in the control group by 3.9-6.7%, respectively [20, 32]. Similar results were obtained for poultry of the meat production direction — Cornish chicks of the cross Smena 8 [33]. A similar effect was observed in the embryos of ducks: injection of glutamine and carbohydrates (sucrose and maltose) *in ovo* led to an increase in live weight at the end of rearing, improved the development of the intestines and pectoral muscles [34].

Bhanja et al. [35] showed that *in ovo* injections of glucose (50 mg per embryo) on day 18 of incubation affected the development of the digestive system and the biochemical profile of the blood of chicks. In 1-day-old chicks from the experimental group, the content of glucose and protein in the blood plasma, the weight of the liver, glandular and muscular stomachs, as well as the small intestine, increased. On day 10 of life, in chicks receiving glucose during the embryonic period, the concentration of glucose and uric acid in the plasma significantly decreased, and the weight of the spleen and small intestine increased [35].

The use of exogenous amino acids in embryogenesis. In poultry, the pectoral muscle tissues serve as the main source of amino acids for gluconeogenesis when there is a lack of energy, which can lead to its atrophy [25, 36, 37]. Under late access to the feed, the development and growth of skeletal muscles are delayed and lags behind until slaughter age [17].

Ohta et al. [38] conducted experiments to evaluate the effect of *in ovo* injections of a mixture of amino acids on their use by embryos. It was shown that in the group receiving amino acids *in ovo* on day 7 of incubation, on day 19, the content of amino acids in the embryo, yolk, albumen, allantoic fluid, and amnion fluid was significantly higher ($p < 0.05$) than in the control (distilled water); in comparison with the control group, the absolute and relative weight of 1-day-old chicks also increased [38].

These results were confirmed when studying the effects of introducing a mixture of amino acids (0.75 ml per embryo) on ducks: when hatching, the mass of chicks was 6.2 higher than in the control; in addition, an increase in the mass of lymphoid organs was noted [39, 40].

In a number of experiments, the effect of individual amino acids was studied. Thus, Coskun et al. [41] demonstrated a positive effect of the introduction of methionine (50 μl per egg) into the amnion of broiler embryos: the relative weight of chicks increased by 2.7% compared to the control. Tahmasebi et al. [42] demonstrated a positive effect of threonine (25 mg per egg) introduced into the amnion on day 14 of incubation, which led to an increase in the growth rate of chicks compared with the control group ($p \leq 0.05$), as well as improvement in the development of organs of the gastrointestinal tract.

Ohta et al. [43] suggested that the concentration of amino acids in eggs, for example, glycine and proline, was insufficient to support embryo development in the final phase of incubation. This is also confirmed by the studies [44], which showed statistically significant differences in body weight in 1-day-old and 3-week-old chicks, if glycine and proline were administered during the embryonic period, compared with the control group.

Tong and Barbul [45] state that arginine is an essential amino acid for embryos, which is mainly due to its role in protein synthesis. Arginine is involved in a number of metabolic pathways, taking part in the formation of various biologically active compounds, which also helps to maximize the development

potential of the embryo by stimulating the secretion of growth hormones. It is known that arginine serves as a substrate for the synthesis of nitric oxide, the rate of oxidation of which during embryonic development is associated with the growth rate of chicks after hatching [46, 47]. In chicks receiving arginine during the embryonic period, after hatching, the growth rate and enzymatic activity of the digestive glands increased, and the morphological development of the organs of the gastrointestinal tract improved [42, 48]. It was shown that the hatchability of eggs was higher in the group where the embryos were injected with arginine and lysine on day 18 of incubation; on day 42 of life, the body weight of the obtained chicks was higher compared to the control group [49]. In other experiments, *in vivo* injections of 0.6% arginine solution contributed to an increase in the concentration of albumin in the blood plasma, the deposition of protein in the pectoral muscles, and, as a consequence, an increase in the growth of pectoral muscles in chicks [50].

Similar effects were observed in other poultry species. After the injection of a 3% solution of arginine into the air chamber of quail embryos, the synchronism in the hatching of chicks increased, the live weight increased on days 7 and 42 of life, and the feed conversion improved compared to the control group [51]. In the experiments on turkeys, feeding *in ovo* with the solution containing 0.7% arginine contributed, on average, to a twofold increase in the activity of pancreatic digestive enzymes (saccharase, maltase, leucyl aminopeptidases) in the small intestine of embryos on day 25 [52]. On day 14 of life of chicks, the activity of these enzymes was 3 times higher than in the control [52].

As it was noted, the effect of *in ovo* feeding may be due to epigenetic mechanisms. It was shown that *in ovo* injections of L-arginine at different times of incubation of chick embryos increased the expression of genes for myoblast determination factors (MyoD) and myogenin in the pectoral muscles of embryos [53]. The authors of the study explain the observed effect by the fact that L-arginine is a precursor of NO [53]. The effect of nitric oxide on the stimulation of the processes of embryonic myogenesis by enhancing the expression of myogenic regulatory factors was shown during *in ovo* injections to chick embryos of NO synthase inhibitors — an enzyme involved in the formation of nitric oxide from L-arginine, or NO donors [54].

The epigenetic effect of exogenous amino acids was noted in the case of *in ovo* administration of sulfur-containing amino acids methionine and cysteine, which increased the expression of genes for insulin-like growth factor (IGF-1) and toll-like receptor 4 (TLR-4) [55].

Features of energy metabolism of poultry embryos and the use of antioxidants and vitamins in embryogenesis. The metabolism of poultry in the embryonic period has some peculiarities and differs from the metabolism in postnatal ontogenesis. This is due to the unique structure of the poultry's egg, in which almost the entire supply of energy sources is yolk triglycerides and partly proteins, while free carbohydrates are extremely small: 0.5% in the yolk and 0.2% in the albumen, of which 98% are glucose [56]. The successful development of the embryo in poultry depends on the delivery of a sufficient amount of lipids (in a certain ratio) from the yolk to the embryo and the metabolic ability of the embryo tissues to utilize them for growth and differentiation. It was estimated that 94% of the total metabolic energy of the embryo during development was generated as a result of fatty acid oxidation [28, 57].

The presence of a large amount of unsaturated fatty acids in the presence of oxygen is fraught with the occurrence of oxidative processes with the formation of oxygen radicals. They lead to damage to embryonic and germinal structures and

the accumulation of toxic compounds. Antioxidants counteract the negative effects of free radicals and thereby protect the embryo from damage [58, 59].

The positive effect of *in ovo* injections of vitamins E (10 mg) and C (3 mg) on the body weight of ducklings after hatching and on their subsequent growth rate was established. The experimental groups were characterized by improved feed conversion [60]. The study of the effect of vitamin E on productivity and immunological parameters of the blood of chicks after hatching revealed the effect of this compound on the development of the immune system in chicks during the rearing period. Injection of vitamin E at a dose of 30 mg resulted in increased resistance to avian influenza and infectious bronchitis. In the experimental groups, an increase in the titers of immunoglobulins IgG, IgM, and IgA was noted [61]. Nowaczewski et al. [62], considering the effect of vitamin C on the hatchability of chicken and duck eggs, found that its positive effect was manifested only in duck eggs. In chicken eggs, injections of vitamin C did not have a significant effect on improving hatchability. In duck eggs, the best hatching results were obtained in experimental groups, regardless of the dose and time of *in ovo* administration of ascorbic acid. On average, the difference in hatchability of duck eggs between the experimental and control groups was 32.5% [62]. Opposite results for chicken eggs were obtained by Zhu et al. [63]: when 11-day-old embryos were injected with ascorbic acid (3 mg/egg), both an improvement in hatchability and an increase in the growth rate of chicks up to 42 days of age were observed. Moreover, the same authors found an increase in the expression of the *IL-4* and *DNMT1* genes and a decrease in *IL-1β*, *Tet 2*, *Tet 3*, and *Gadd 45β* ($p < 0.05$) in the spleen tissues of 21-day-old chicks, which received ascorbic acid on day 11 of incubation [63]. Apparently, the effect of antioxidants depends on the characteristics of the fatty acid composition of the egg and the content of endogenous antioxidants.

During the last 2-3 days of incubation, due to the high energy intensity of the hatching process and the relatively low availability of oxygen, fatty acids cannot provide the embryo with all the necessary energy [13]. As a result, the embryo switches to anaerobic glucose catabolism, the intensity of which depends on the amount of glucose stored in the form of glycogen of liver, kidney and muscles and generated during gluconeogenesis from amino acids, glycerol, and lactate [23, 49].

One of the substances that stimulate the oxidation of fatty acids for energy production is L-carnitine, which belongs to the group of biologically active compounds and plays an important role in energy metabolism during embryogenesis, participating in the transfer of acyl groups of fatty acids from the yolk to the tissues of an embryo. Poultry embryos have a limited ability to synthesize L-carnitine during incubation. The studies on the effect of exogenous sources of L-carnitine gave ambivalent results. In some experiments, the introduction of L-carnitine before incubation at doses from 2 to 12 mg per egg did not significantly affect the postembryonic development of chicks [18]. Elevating the dose of L-carnitine increased the hatching time and decreased the hatchability; the authors do not provide an explanation of the mechanism that caused the decrease in hatchability [18]. In other studies, the use of L-carnitine *in ovo* did not decrease hatchability and did not increase the hatching period of chicks [64]. At the same time, there was an increase in the absolute and relative weight of chicks at hatching, the content of glycogen in the liver and pectoral muscle, and insulin-like growth factor in the blood plasma [64]. It was also shown that the injection of L-carnitine on day 14 of incubation significantly increased hatchability, increased the growth rate of chicks, and improved feed conversion [65]. Similar results were obtained in the authors' studies. As a result of *in ovo* injection of L-carnitine in an amount of 2-3 mg per egg, hatchability increased, in 1-day-old chicks, there was a statistically

significant increase in weight, a significant decrease in glucose concentration, and an increase in the activity of lactate dehydrogenase in the blood serum, which indicates increased utilization of glucose under anaerobic conditions during the hatching period [66, 67].

It should be noted that the dosage of L-carnitine used in the studies varies greatly. Apparently, a clear understanding of the rate of carnitine synthesis during embryogenesis, as well as control of its initial content and correction in the case of deviation from the norm, are required.

Thus, the prenatal period in the development of poultry is an extremely important and critical period. Egg feeding technology can help the chick successfully overcome this stage and fully realize its genetic growth potential. The increase in the growth rate of poultry is due to the ability of the intestines to better digest and assimilate food. This can be achieved by *in ovo* administration of compounds that stimulate the functional activity of the cells of the gastrointestinal tract. In the first week after hatching, metabolic and physiological changes occur in the organism of the chicks. Faster adaptation of the gastrointestinal tract to exogenous food is essential for growth and increased vitality. It is also important that prenatal feeding has an epigenetic effect, inducing the expression of genes, the products of which are involved in the main metabolic pathways and processes in tissues and organs and thereby affect productivity. Consequently, feeding in eggs can be a tool to significantly improve hatchability and the vitality of chicks.

Despite a lot of conducted research, the method of prenatal feeding has not yet found industrial application. The data on dosages of exogenous substances vary significantly, as well as the timing of their use. To select the optimal dose, it is necessary to control the carbohydrate, amino acid, and vitamin composition of the embryo. This, in turn, requires establishing the norms for the content of such compounds in the embryo and developing the technology for the rapid accurate determination and correction of the corresponding parameters in the hatching egg. To implement the discussed technology into practice, research is required using existing automated systems [68, 69].

Influence of *in ovo* administered substances on hatchability and the growth rate of chicks

Active substance	Incubation period, days/dose per embryo	Influence		Reference
		on hatchability	on growth	
Glucose, maltose, dextrin	18/25 mg	+	+	[22]
Dextrin, hydroxymethyl butyrate	18/0.7 ml, 0.4% solution	No data	+	[19]
Glucose, dextrin	17/0.5 ml 10 % solution	No influence	+	[32]
Glucose	18/50 mg	No influence	+	[35]
Mixture of amino acids	7/0.7 ml	–	+	[43]
Mixture of amino acids	7/53 mg	+	+	[38]
Methionine	16/50 μl	–	+	[41]
Threonine	14/25 mg	No data	+	[42]
Glycine + proline	14/5.8 ml	–	+	[44]
Arginine	17.5/0,6 mg	No data	+	[50]
Arginine	0/0.5 мл, 3% solution	+	+	[51]
Arginine	21/1,5 ml, 0.7% solution	No influence	+	[52]
Methionine + cysteine	17.5/6.3 mg	No influence	+	[55]
Ascorbic acid	11/3 mg	+	+	[63]
Ascorbic acid	17/3 mg	–	No data	[62]
Vitamin E	12/10 mg	+	+	[60]
Vitamin E	14/15-30 mg	+	+	[61]
Folic acid	11/100-150 μg	+	+	[18]
Carnitine	0/8 mg	–	No influence	[64]
Carnitine	18/25-100 μl	No influence	+	[65]
Carnitine	14/4-12 mg	+	+	[66]
Carnitine	17/3 mg	+	+	[22]

N o t e. "+" – improvement, "–" – deterioration, "no influence" — the indicator remained unchanged.

Moreover, the cost-effectiveness of such a technology is not clear.

Proceeding from the fact that its main significant indicators are hatchability and the growth rate of chicks, the authors briefly summarized the data available in the special literature on this topic (Table).

Thus, despite the fact that the overwhelming majority of researchers declare the positive effect of prenatal feeding on the growth rate of poultry, many questions remain unresolved and controversial. The effect on the viability of embryos after their exposure to exogenous substances is ambiguous; their dosage and delivery time to the embryo are not clear. Establishing the norms for the content of such exogenous substances in the egg and studying the physiological mechanisms of the response of embryos after exposure to them remains the most important task, without solving which it is impossible to successfully regulate the productive qualities of poultry at the embryonic stage.

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